

PROJECT NUMBER: 2525
PROJECT TITLE: Tobacco Chemistry
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I. NATURAL PRODUCTS CHEMISTRY

- A. Objective: To isolate, identify and/or analyze natural compounds with major emphasis on tobacco and tobacco products.
- B. Results: Additional quantities of Bright tobacco extract Fr IV were separated into four fractions by vacuum liquid chromatography (VLC) on ODS-silica. Analytical LC profiling of the VLC fractions on an aminopropylsilica column (Zorbax NH₂) was conducted.
- C. Plans: Continue fractionation with emphasis on optimizing production.
- D. References:
 - 1. Tafur, S. Notebook No. 8417.
 - 2. Core, M. Notebook No. 8608.

II. LOW NICOTINE

- A. Objective: To assist in characterizing unextracted nicotine.
- B. Results: It has been reported by several investigators that nicotine transfers into smoke without a change in optical purity. In order to verify this cigarettes were obtained and the nicotine isolated from the filler and from the smoke condensate. The general procedure for the isolation of nicotine involves extraction with 0.5N HCl, partition with dichloromethane, basification of the aqueous layer with KOH, partition with dichloromethane, evaporation of the solvent (MeCl₂, to yield the crude alkaloids), Kugelrohr distillation (to yield crude nicotine), and HPLC (silica, hexane/acetone/isopropanol/triethylamine, 65/30/5/1.5) to give purified nicotine. Prior to measuring the optical rotation, the nicotine was subjected to a final Kugelrohr distillation. The following yields of nicotine were obtained:

cig. #	quantity	wt. condensate (g)	wt. alkaloids (g)	wt. nicotine (crude, g)
5405	510	5.3	0.5	0.2
5406	3,000	29	2.5	0.2
5405*	500	---	0.8	0.5
5406*	1,500	---	0.4	0.1

5405 are control cigarettes with casing (filtered, 1.13mg nic/c).
5406 are ART cigarettes (filtered, 0.15mg nic/c).

*extraction of filler

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A reversed phase HPLC system for the determination of nicotine in aqueous solution is being optimized for a nicotine diffusion study. The lower limit of detection was found to be about 1 ng of nicotine per injection.

C. Plans: Continue measurement of optical rotations. Measure diffusion constant of nicotine. Start model study of the interaction of nicotine and protein.

D. References:

1. Izac, R. Notebook No. 8379.
2. Core, M. Notebook No. 8608.

III. GREENHOUSE STUDIES

A. Objective: To maintain the R&D greenhouses, to conduct plant research studies and to provide greenhouse-grown tobacco materials for support of other R&D programs.

B. Results: Group nine, Burley 21 plants are being harvested at different intervals after topping to determine the optimum enzyme yields. The group ten plants have been set up and transplanted.

The total amount of dry weight ^{14}C plant material was 605g including 201g of leaf material. The average specific activity was $274\mu\text{Ci/g}$ of tobacco. The amount of $^{14}\text{CO}_2$ injected into the chamber during Run #12 was 540.8 grams.

Total alkaloid and sugar analyses by AR have been completed for 158 S. C. Tests, 180 Speight Seed Farms 1987 Breeding lines, and 180 flue cured tobacco samples from the 1987 Northrup King Seed Co. Breeding line test.

Manicured 2R1 filler was sprayed with $500\mu\text{Ci}$ of ^{14}C glycerol and a total of 34 hand made cigarettes were prepared.

A solution of sodium $^{13}\text{C-}\beta\text{-methylvalerate}$ was stem fed to a Coker 319 leaf. After uptake of the solution the leaf developed the aroma of $\beta\text{-methylvaleric acid}$. The leaf was oven dried and submitted for NMR analysis.

C. Plans: Continue to produce fresh root tissue by hydroponic culture. Isolate sucrose ester fraction from leaf fed $\beta\text{-methylvaleric acid}$.

D. References:

1. Bass, R. Notebook No. 8495.
2. Izac, R. Notebook No. 8379.

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IV. SS/MS FLAVOR DISTRIBUTION

A. Objective: Study the SS/MS distribution of vanillin/gluco-vanillin.

B. Results: Vanillin standard solutions were prepared and given to J. Naworal (Project 2501) for development of an analytical method. TPM pads were spiked with vanillin at three different levels and then extracted with acetone to determine extraction efficiency. Five PM Blues cigarettes were smoked on a SAVA smoking machine for the collection of MS and SS. Acetone extracts of MS and SS TPM pad, SS cold trap, SS chamber wash and butt with filter were obtained and provided to J. Naworal for analysis.

C. Plans: Continue smoking and analysis.

E. References: Tafur, S. Notebook No. 8490.

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